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For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 01/075164 A3

(54) Title: RNA SEQUENCE-SPECIFIC MEDIATORS OF RNA INTERFERENCE

(57) Abstract: The present invention relates to a *Drosophila* in vitro system which was used to demonstrate that dsRNA is processed to RNA segments 21-23 nucleotides (nt) in length. Furthermore, when these 21-23 nt fragments are purified and added back to *Drosophila* extracts, they mediate RNA interference in the absence of long dsRNA. Thus, these 21-23 nt fragments are the sequence-specific mediators of RNA degradation. A molecular signal, which may be their specific length, must be present in these 21-23 nt fragments to recruit cellular factors involved in RNAi. This present invention encompasses these 21-23 nt fragments and their use for specifically inactivating gene function. The use of these fragments (or chemically synthesized oligonucleotides of the same or similar nature) enables the targeting of specific mRNAs for degradation in mammalian cells, where the use of long dsRNAs to elicit RNAi is usually not practical, presumably because of the deleterious effects of the interferon response. This specific targeting of a particular gene function is useful in functional genomic and therapeutic applications.

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INTERNATIONAL SEARCH REPORT

In national Application No
PCT/US 01/10188

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C12Q1/68 C12N15/10

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C12Q C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, WPI Data, BIOSIS, MEDLINE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	TUSCHL THOMAS ET AL: "Targeted mRNA degradation by double-stranded RNA in vitro"	6-8
Y	GENES AND DEVELOPMENT, COLD SPRING HARBOR LABORATORY PRESS, NEW YORK, US, vol. 13, no. 24, 15 December 1999 (1999-12-15), pages 3191-3197, XP002183118 ISSN: 0890-9369 cited in the application the whole document	9-11, 13-15, 17-25, 28-33, 38-40, 44, 45
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☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

* Special categories of cited documents:

- *A* document defining the general state of the art which is not considered to be of particular relevance
- *E* earlier document but published on or after the international filing date
- *L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- *O* document referring to an oral disclosure, use, exhibition or other means
- *P* document published prior to the international filing date but later than the priority date claimed

- *T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- *X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- *Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
- *&* document member of the same patent family

Date of the actual completion of the international search

9 October 2002

Date of mailing of the international search report

18/10/2002

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INTERNATIONAL SEARCH REPORT

Int. Application No

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	HAMMOND SCOTT M ET AL: "An RNA-directed nuclease mediates post-transcriptional gene silencing in <i>Drosophila</i> cells" NATURE, MACMILLAN JOURNALS LTD. LONDON, GB, vol. 404, no. 6775, 16 March 2000 (2000-03-16), pages 293-296, XP002183123 ISSN: 0028-0836	46, 47
Y	the whole document	9-11, 13-15, 17-25, 28-33, 38-40, 44, 45
A	NGÔ ET AL: "Double-stranded RNA induces mRNA degradation in <i>Trypanosoma brucei</i> " PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF USA, NATIONAL ACADEMY OF SCIENCE. WASHINGTON, US, vol. 95, December 1998 (1998-12), pages 14687-14692, XP002138442 ISSN: 0027-8424 the whole document	9
A	HAMILTON ANDREW J ET AL: "A species of small antisense RNA in posttranscriptional gene silencing in plants" SCIENCE, AMERICAN ASSOCIATION FOR THE ADVANCEMENT OF SCIENCE, US, vol. 286, no. 5441, 29 October 1999 (1999-10-29), pages 950-952, XP002149064 ISSN: 0036-8075 cited in the application the whole document	9
P, X	ZAMORE PHILLIP D ET AL: "RNAi: Double-stranded RNA directs the ATP-dependent cleavage of mRNA at 21 to 23 nucleotide intervals" CELL, CELL PRESS, CAMBRIDGE, NA, US, vol. 101, no. 1, 31 March 2000 (2000-03-31), pages 25-33, XP002208683 ISSN: 0092-8674 the whole document	9-11, 13-15, 17-25, 28-33, 38-40, 44-47
E	WO 01 68836 A (BEACH DAVID ; CAUDY AMY (US); HAMMOND SCOTT (US); BERNSTEIN EMILY () 20 September 2001 (2001-09-20) page 6, line 20 - line 34 page 10, line 4 -page 11, line 22	9-11, 13-15, 46, 47

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 01/10188

Box I Observations where certain claims were found unsearchable (Continuation of Item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☒ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
see FURTHER INFORMATION sheet PCT/ISA/210

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of Item 2 of first sheet)

This International Searching Authority found multiple inventions in this International application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.2

Claims Nos.: 1-5,12, 16,26,27,35-37,42,43,48-50 (completely),
6,9,11,13,15,20,22,30,31,33,46,47 (all partially)

1) Product claims 1-5,12,16,35-37,43,48-50 relate to RNA molecules solely characterised in that they mediate RNA interference. This sole functional feature, however, is not sufficient to characterise the claimed molecules so as to allow the skilled person to clearly and unambiguously understand the scope of the claims. Moreover, the skilled person is given no guidance, either in the claims or in the description, as to any general technical feature of the claimed RNA molecules which could allow him/her to understand, with no undue burden, which molecules fall and which do not fall within the definition of the claims.

Claims 26 and 27 relate to a knockdown cell obtained by treating it according to the method of claim 23. However, the sole indication that a cell has been obtained by treating it with a short RNA molecule with no indication as to features of the cell, such as e.g. the kind of cell and the kind of gene which has been silenced does not allow the skilled person to clearly and unambiguously understand the scope of the claim. Similar arguments apply to claim 42, which relates to a gene solely characterised by means of the method used for its identification.

Thus, the aforementioned claims 1-5,12,16,35-37,43,48-50, 26, 27 and 42 lack clarity (Art. 6 PCT) to such an extent as to render a meaningful search with respect to their subject-matter impossible.

2) Claims 6, 9, 13, 20, 30,46 relate broadly to a soluble extract that mediates RNA interference. However, the description only provides indications as to a *Drosophila* embryo extract having such a capability (see p. 22, l. 1-10, p. 28, l. 9, p. 32, l. 10, p. 36, l. 14, p. 40, l. 18) and is completely silent as to any general rule that the skilled person could apply to identify, with no undue burden, other suitable extracts. The description discloses that two other lysates have been tested, namely wheat germ extract and rabbit reticulocyte lysate (see p. 28): the former showed no effect whatsoever, the latter only had a non-specific effect, not linked to RNA interference (p. 28, l. 18-20). Hence these claims lack support (Art. 6 PCT) to such an extent as to render a meaningful search of their subject-matter impossible. The search with respect to these claims has thus been limited to soluble extracts derived from *Drosophila* embryos.

The applicant's attention is drawn to the fact that claims, or parts of claims, relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.

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INTERNATIONAL SEARCH REPORT

Information on patent family members

Int. Application No

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Patent document cited in search report		Publication date	Patent family member(s)	Publication date
WO 0168836-	A	20-09-2001	AU 4579301 A	24-09-2001
			WO 0168836 A2	20-09-2001

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